

EARLY DEVELOPMENT OF FLORAL ORGANS AND EMBRYONIC STRUCTURES OF SCROPHULARIA MARYLANDICA

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(WITH PLATES XXVII-XXIX)

The material used in this study was collected at Evanston, Illinois, in the summers of 1909 and 1915, and Bloomington, Indiana, in the summer of 1910. It was killed in medium chromic acetic acid and preserved in 70 per cent alcohol. The paraffin method was used, and difficulty was encountered because of the hardened walls of the seed case. Consequently, the walls of the seed case were dissected away, leaving the ovules attached to the axile placentae, and the difficulty was greatly lessened, although not entirely obviated because of the thick and hardened testa which remained.

Development of floral organs

The order of floral development in *Scrophularia marylandica* L. was found to be calyx, stamens, corolla, and pistil. It seems to be assumed that in the majority of cyclic flowers the parts appear in acropetal succession, namely, sepals, petals, stamens, and carpels. In *Astilbe* WEBB has observed that the order of succession of the floral parts is sepals, inner stamens, carpels, outer stamens, and petals. The origin of the petals and the stamens in *S. marylandica* is very similar to that of the flowers of the Primulaceae, in that the primordia of the petals appear after those of the stamens, and each petal apparently comes from the dorsal surface of a young stamen.

Megasporangium

The anatropous ovules arise from central placentae and develop a single integument (fig. 10). Only one layer of cells of the nucellus incloses the mother cell at this stage (fig. 11), but a marked change soon occurs, due to the growth of the integument. This

tegumentary tissue fully surrounds the megaspore mother cell, even as early as the time of the reduction division (fig. 12) of the mother cell. The greatly elongated mother cell forms the four potential megaspores (fig. 13), of which the three potential micropylar megaspores soon degenerate, while the fourth or functioning one forms the embryo sac.

The nuclei of the three degenerating cells (fig. 14) soon disappear, while that of the functional megaspore is clearly visible. In degenerating, the potential megaspore cell next to the functional megaspore disappears first; for a time it forms a sort of cap upon the functional megaspore cell. The three degenerating cells stain very heavily, while the megaspore cell stains lightly. These degenerating cells, which stain deeply with safranin, are last observed as a strip of red above the functional megaspore.

Nucellus

While the megaspore mother cell is being formed (fig. 11), a layer of nucellar tissue envelops it. The rapidly developing integument soon surrounds the nucellus and its mother cell. The cells of the nucellus are long and narrow, and their transverse walls are usually oblique.

A similar tissue arises in many of the other species of the Scrophulariaceae. BALICKA-IWANOWSKI (1) calls it "nucelle," and figures such a tissue in *Uroskineria spectabilis*, *Barisia alpina*, *Pedicularis palustris*, *Klugia notoniana*, *Campanula rotundifolia*, and *Marina longifolia*.

The contents of these cells stain with Delafield's haematoxylin less heavily than the four megaspore cells, and less than the cells immediately surrounding them. As the embryo sac forms, it pushes its way through the micropylar end of the nucellus layer, and when the embryo sac is fully formed the nucellus is found surrounding only the chalazal end of the sac. The nucellus disappears while the endosperm is being formed within the embryo sac. It is still apparent when the first division of the endosperm nucleus takes place, and also until after four cells of the endosperm have been formed. Cells of the nucellus were not observed to be present in later developments of the endosperm (fig. 25).

Tapetal layer

The tapetal layer appears first as a ring or band of cells surrounding the nucellus (fig. 12). The cells of the tapetal layer divide only anticlinally, and later come to form a layer of cells completely surrounding the embryo sac. The cells of this layer lie with their longer axes perpendicular to the embryo sac, while the cells of the nucellus layer within them lie with their longer axes parallel with the embryo sac. This layer of cells is more persistent than the nucellus, and is observed even after most of the endosperm is formed. When the embryo approaches maturity no traces remain of the tapetal layer. These cells possess large nuclei and are well filled with protoplasmic contents. In all of the species of this group as figured by BALICKA-IWANOWSKI, this tapetal layer is shown. The extent to which the tapetal layer incloses the embryo sac varies. In *S. marylandica* the tapetum almost surrounds the embryo sac, or at least to a greater extent than it does in any of the species as figured by BALICKA-IWANOWSKI.

Nutritive tissue

In some species, as *Scrophularia vernalis* and *Scoparia dulcis*, the micropylar end of the tapetal layer is composed of many cells and is called nutritive tissue. In *Digitalis purpurea*, *Linaria Cymbalaria*, and *Torenia Deli* no such tissue is found. At the chalazal end, where the tapetum leaves off, the nutritive tissue completely encircles the lower extremity of the embryo sac in *Scrophularia marylandica* (fig. 22) and in *Linaria Cymbalaria*. In *Scoparia dulcis* no chalazal or micropylar nutritive tissue is observed, and in *Torenia Deli* this tissue is very scanty. The cell walls of the nutritive tissue stain deeply with Delafield's haematoxylin, and the nuclei are less prominent than they are in the surrounding tissues. This seems to indicate that these cells are chemically different from the surrounding tissues.

Embryo sac

The embryo sac develops from the functioning megaspore, and comes to occupy the space formerly occupied by the three potential megaspores. The "nucelle" layer of BALICKA-IWANOWSKI,

judging from the number of cells, does not appear to divide and form new cells after the first division of the megaspore mother cell, but rather, the megaspore by dividing first into two cells (fig. 15), and then into four cells (fig. 16), develops in such a manner as to protrude from the region of the "nucelle." The embryo sac is long and narrow in its chalazal part, and is enlarged in the micropylar end. After the embryo sac reaches the 4-cell stage, as in fig. 16, the nuclei migrate, and the two micropylar nuclei go to the micropylar end of the sac and arrange themselves one on each side of the sac. The nucleus closest to the micropylar end of the embryo sac now divides to form two nuclei, from which the cells of the pear-shaped synergids arise. The other micropylar nucleus divides to form the egg nucleus and the micropylar polar nucleus. The nucleus of the egg had about the same diameter as the synergid nuclei when observed, while the polar nucleus had about twice the diameter of any of the other three micropylar nuclei.

The other two of the four nuclei migrate to the chalazal end of the sac. One of the two chalazal nuclei is found almost at the very tip end of the embryo sac, while the other is located just above it.

It is here that both of these nuclei divide to form the four antipodal nuclei. When the two nuclei divide, their spindles form with the poles toward the ends of the embryo sac rather than crosswise. The three lower nuclei (fig. 18) later become the three degenerating antipodal cells. They degenerate rapidly while the upper nucleus migrates toward the center of the embryo sac, and as it migrates, it greatly enlarges and comes to be as large as the polar nucleus from the micropylar end of the sac. The micropylar polar nucleus and the chalazal polar nucleus now fuse. In the stage before fusion, the nucleoli of both are very prominent (fig. 21), and the nucleus of each is somewhat more than two diameters of the nuclei in the synergids. The large secondary endosperm nucleus now migrates upward toward the central part of the embryo sac, where it is found later, gorged with food.

In *Digitalis purpurea* BALICKA-IWANOWSKI observed remnants of the antipodals, while in *Linaria Cymbalaria* the antipodals are distinct and persist until the complete formation of the chalazal

haustoria. WESTERMEYER, as reported by BALICKA-IWANOWSKI, says that the antipodals of the Scrophulariaceae are particularly difficult to verify.

Embryo

After fertilization the fertilized egg recedes somewhat from the micropylar end of the embryo sac. The first division takes place transversely. It is followed by a longitudinal division in the chalazal cell only. The micropylar cell next divides transversely, giving rise to the first suspensor cells. The two cells of the embryo now divide to form the quadrant stage (fig. 28). The two suspensor cells divide (fig. 34) to form a linear row of four cells, which was the largest number observed. The embryo continues to develop in the normal way to form the mature dicotyledonous type (fig. 35). At no time in its development was the embryo observed to be attached to the micropylar end of the embryo sac by an enlarged basal cell, common in dicotyledons. The embryo of *Plantago maritima* is very similar to the one just described.

Haustoria

In the lower central part of the embryo sac, the secondary endosperm nucleus divides to form endosperm. When the endosperm nucleus has divided to form four cells (fig. 24), the two chalazal cells assume the character of haustoria. The nuclei in them at first (figs. 24, 25) are very prominent, but later seem to degenerate (fig. 29), and when the embryo is fully formed the haustoria themselves entirely disappear.

When the two remaining endosperm cells divide further, they produce four prolongations, which are the micropylar haustoria. These four haustoria are cut off when the endosperm has four cells in cross-section (fig. 25). They may be regarded as absorbing and conducting organs for the transport of food to the rapidly developing endosperm from the surrounding tissues. They are gorged with food matter and react strongly to Delafield's haematoxylin stain. Thickenings of the protoplasm occur in the older haustoria, and may be considered as degenerating nuclei. The nutritive cells of the ovule at the chalazal end of the embryo sac, it may be inferred, are disintegrated by enzymes secreted by the haustoria. Of the

micropylar and the chalazal haustoria, the latter are the larger. As there are no nutritive tissues at the micropylar end of the sac, this accounts for the micropylar haustoria being less developed than those of the chalazal end of the embryo sac. The chalazal haustoria also have a greater region of nutritive tissue from which to absorb. The haustoria attain their greatest development when the endosperm is being formed most rapidly. As the embryo reaches maturity only traces of the haustoria remain.

In the Rhinanthaeae and other members of the Scrophulariaceae micropylar and chalazal haustoria appear to be quite constant characteristics. The form of the haustoria and the extent to which they are developed vary considerably among the different species, for in *Melampyrum memorosum* they are very arborescent, while in some of the other species only rudimentary haustoria appear. In *Scoparia* no haustoria are noticeable. BALICKA-IWANOWSKI is inclined to think that the micropylar haustoria are not transformed synergids, while SCHLOTTERBECK, to whom reference is made, takes the opposite view. The writer interprets the micropylar and the chalazal haustoria as transformed endosperm cells.

Endosperm

The secondary endosperm nucleus migrates to the lower central part of the embryo sac, and endosperm formation begins with the division of the endosperm nucleus. The secondary endosperm nucleus divides transversely to form the nuclei, and almost immediately a cell wall is formed between them. Each of the two endosperm cells now formed divides longitudinally, and thus four cells are formed. At this stage (fig. 24) the egg and the two synergids were still observed at the micropylar end. The lower two of the above four cells form the haustoria, while the upper two divide transversely and then longitudinally to form eight endosperm cells (fig. 25). At this stage four haustoria are observed at the micropylar end of the embryo sac. They are put out as prolongations of the four micropylar endosperm cells. One cannot regard the micropylar haustoria as transformed synergids. Rapid nuclear division now ensues without reference to these rows of endosperm cells. Endosperm formation takes place before the first division of the egg. As the embryo matures, the surrounding endosperm storage cells

greatly thicken their walls, and their contents consist now of crystalloid protein materials (fig. 36) along with other stored foods.

When the endosperm is formed, it first occupies only the space outlined by the early stage of the embryo sac (fig. 22). As the development of the endosperm proceeds, enzymotic processes set in and the tapetal cells, along with several layers of cells just without, are absorbed and changed into endosperm structure. When the endosperm consists of only two layers of cells throughout the length of the embryo sac (figs. 29, 30, 32), several layers of cells are evident in the surrounding tissues. At a later stage (fig. 33) 8-10 layers of cells were observed in the cross-section of the endosperm, and then not so many layers (3-6) of cells were found in the surrounding tissues. It is evident that the endosperm tissue is being increased at the expense of the surrounding layers of cells. The egg up to this time has not divided. When the egg divides, the endosperm (fig. 26) in cross-section is many layers thick, while the remaining cells of the surrounding tissues have collapsed and become compressed into a thin layer. The cells at the end of the embryo sac are not so greatly changed as are the cells surrounding the sides, and even when the embryo is mature, the cells at the ends of the sac are still noticeable but are slightly compressed. At about the time of the quadrant stage of the embryo (fig. 28), enzymes are secreted and the endosperm tissue surrounding the embryo, extending greatly toward the chalazal end, becomes disintegrated. At this stage the embryo grows rapidly, and soon the cotyledons differentiate. The embryo is now found lying within the endosperm.

The cells of the endosperm are of two types. Those which immediately surround the embryo are long and narrow and mostly devoid of all protein matter, their relation to the embryo evidently leading to a loss of all their cell contents. The cells surrounding these have greatly thickened cell walls and are gorged with protein crystals (fig. 36) and other stored foods. The endosperm is entirely surrounded by a thick hardened coat which stains heavily with Delafield's haematoxylin, and from all appearances serves to protect the seed from loss of moisture. This coat is the testa. Remnants of the single integument persist as an outer covering composed of two or more layers of cells.

One of the rows of cells of the integument has become modified and now appears as a loosely scattered row of cells which connects the outermost layers of cells with the inner thickened hardened layer. Compressed cells, the remnants of the micropylar and the chalazal ends of the embryo sac at the time of fertilization, persist in the seed at each end just outside of the endosperm. The layers of cells covering the seed of various members of the Scrophulariaceae are described by BACHMAN. The number of layers of the testa of the seeds of the 128 species which he describes varies from one to four.

Summary

1. The order of the development of the floral parts of *Scrophularia marylandica* is calyx, stamens, corolla, and pistil. The stamens and the corolla arise from a common outgrowth.

2. The archesporium of the megaspore consists of a single hypodermal cell, which functions as a megaspore mother cell.

3. The megaspore mother cell by two successive divisions gives rise to an axial row of four potential megaspores; the embryo sac arises from the chalazal one, while the other three degenerate.

4. The mature embryo sac contains one egg, two large synergids, an endosperm nucleus, and three antipodal nuclei which soon degenerate.

5. A secondary endosperm nucleus, which grows larger as it migrates toward the egg, was observed. A polar nucleus from the chalazal end was seen to fuse with a polar nucleus from the micropylar end.

6. The first division of the fertilized egg is transverse, and is followed by a longitudinal division of the chalazal nucleus, while the other nucleus fails to divide until later.

7. The nucellus consists of a single layer of cells which surrounds the megaspore.

8. A tapetal layer develops around the embryo sac. It is one cell thick and begins to form at the time the megaspore mother cell divides. At the chalazal end an extensive nutritive layer is formed at the same time.

9. Two well developed haustoria are formed at the chalazal end of the embryo sac. Four less developed ones are formed at the micropylar end of the embryo sac.

10. Only a single thickened integument is found.

11. Endosperm formation takes place before the fertilized egg divides. Endosperm cells separate the fertilized egg from the micropylar end of the embryo sac.

12. The embryo develops a short suspensor which disappears as the embryo matures.

13. The mature seed consists of an embryo surrounded by thickened endosperm cells greatly gorged with crystalline protein and other food matter.

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EXPLANATION OF PLATES XXVII-XXIX

Abbreviations used are as follows: *a*, antipodals; *b*, bract; *c*, cotyledon; *co*, corolla; *d*, degenerate nucleus; *e*, embryo; *f*, funiculus; *h*, haustoria; *i*, integument; *j*, endosperm; *k*, micropyle; *l*, egg; *m*, megaspore; *mc*, megaspore mother cell; *n*, nucellus; *o*, ovule; *oc*, ovary cavity; *p*, pistil; *pn*, polar nucleus; *q*, placenta; *r*, rudimentary flower; *s*, stamen; *st*, stigma; *sa*, stem apex; *t*, tapetum; *u*, protein crystals; *v*, testa; *w*, synergid; *x*, calyx; *y*, suspensor; *z*, nutritive tissues.

All of the figures were outlined by means of an Abbé camera lucida on a level with the stage of the microscope. The details were drawn in freehand. The microscope used was a Bausch and Lomb with a triple nosepiece. The following combinations were used: figs. 1-10, no. 1 ocular and 16 mm. objective; figs. 11-19, 21, 27, 31, 34, and 36, no. 8 ocular and 2 mm. objective; fig. 22, no. 8 ocular and 4 mm. objective; figs. 26, 32, 33, and 35, no. 8 ocular and 16 mm. objective; figs. 23, 24, and 25, no. 8 ocular and 3 mm. objective; fig. 20, no. 12.5 ocular and 2 mm. objective. In all of the figures the micropylar end is toward the top of the plate. Figs. 22, 24, 25, and 35 are each reconstructed from several sections.

FIG. 1.—Longitudinal section of stem apex and young bract before any differentiation has taken place.

FIG. 2.—Similar section of flower with bract, showing calyx being differentiated.

FIG. 3.—Similar section of flower showing bract, calyx, and stamens.

FIG. 4.—Similar section of flower, with calyx and stamens; petals and pistil appearing.

- FIGS. 5, 6.—Parts in fig. 4 at an older stage.
- FIG. 7.—Ovary cavity and stigma being differentiated.
- FIGS. 8, 9.—Showing common origin of stamens and petals, differentiation of ovary, and relation of floral parts.
- FIG. 10.—Section showing pistil and ovules on axile placenta.
- FIG. 11.—Young ovule showing archesporial mother cell and nucellus, also integument.
- FIG. 12.—Young ovule showing megaspore mother cell.
- FIG. 13.—Axial row of 4 megaspores; nucellus, tapetum, and integument also shown.
- FIG. 14.—Axial row of 4 megaspores; 3 micropylar ones degenerating.
- FIG. 15.—Two-nucleate embryo sac.
- FIG. 16.—Four-nucleate embryo sac.
- FIG. 17.—Micropylar end of mature embryo sac.
- FIG. 18.—Chalazal end of mature embryo sac.
- FIG. 19.—Embryo sac showing antipodal polar nucleus, two synergids, egg, and micropylar polar nucleus.
- FIG. 20.—Two synergids and egg.
- FIG. 21.—Fusion of 2 polar nuclei.
- FIG. 22.—Ovule just before fertilization and its relation to nucellus, tapetum, and nutritive tissue; funiculus also indicated.
- FIG. 23.—First division of endosperm nucleus forming 2 cells.
- FIG. 24.—Four cells of endosperm; 2 chalazal ones later form haustoria.
- FIG. 25.—Two chalazal haustoria; the 4 micropylar haustoria and the 8 endosperm cells separating the haustoria.
- FIG. 26.—The 3-celled embryo in surrounding endosperm cells in longitudinal section.
- FIG. 27.—The 3-celled embryo; single cell later forms suspensor, while the 2 cells later form embryo.
- FIG. 28.—Cells dividing to form quadrant stage; 2 suspensor cells shown.
- FIG. 29.—Relation of chalazal haustoria to nutritive tissue; tapetum and endosperm cells.
- FIG. 30.—Relation of micropylar haustoria to tapetum.
- FIG. 31.—Cross-section through central region of embryo sac showing endosperm division into 2 rows of cells throughout the sac.
- FIG. 32.—Outline drawing showing micropyle, funiculus, and relation of the 2 rows of endosperm to rest of ovule.
- FIG. 33.—Later stage showing increase of endosperm in proportion to rest of ovule.
- FIG. 34.—Young embryo showing the 4 cells in suspensor.
- FIG. 35.—Cross-section through mature seed showing embryo, thickened endosperm cells filled with protein, and remains of integument which now functions as the testa.
- FIG. 36.—Enlarged drawing of thick-walled protein-filled endosperm cells.